

We claim:

1. A method for producing amplified RNA (aRNA), said method comprising
 - a) reverse transcribing an RNA template using a promoter-primer complex
 - 5 and an RNA dependent DNA polymerase to produce a first strand cDNA;
 - b) optionally treating the reverse transcription product with RNase H enzymatic activity;
 - c) producing a second strand cDNA complementary to said first strand cDNA using a DNA dependent polymerase, optionally in the presence of random
 - 10 primers to prime synthesis of said second strand cDNA; and
 - d) producing amplified RNA from the eluted double stranded cDNA by *in vitro* transcription using a DNA dependent RNA polymerase which initiates transcription from the primer of said promoter-primer complex;wherein after c) and before d) above, after d) above, or both, the product
 - 15 produced by c) or d) is purified by contacting said product with a solid phase which binds nucleic acids followed by eluting bound nucleic acids from said solid phase in an elution volume of less than 50 microliters.
-
2. A method of producing amplified RNA (aRNA), said method comprising
 - 20 a) reverse transcribing an RNA template using a promoter-primer complex and an RNA dependent DNA polymerase to produce a first strand cDNA in a reaction time of 45 minutes or less;
 - b) optionally treating the reverse transcription product with RNase H enzymatic activity;
 - 25 c) producing a second strand cDNA complementary to said first strand cDNA using a DNA dependent polymerase, optionally in the presence of random primers to prime synthesis of said second strand cDNA, in a reaction time of 45 minutes or less; and

d) producing amplified RNA from the double stranded cDNA by *in vitro* transcription using a DNA dependent RNA polymerase which initiates transcription from the primer of said promoter-primer complex;

5 wherein after c) and before d) above, after d) above, or both, the product produced by c) or d) is purified by contacting said product with a solid phase which binds nucleic acids followed by eluting bound nucleic acids from said solid phase in an elution volume of less than 50 microliters.

10 3. The method of claim 1 or 2 wherein said RNA template is mRNA.

4. The method of claim 1 or 2 wherein said RNA template is in a cellular mRNA preparation.

15 5. The method of claim 1 or 2 wherein said promoter-primer complex comprises an oligo or poly dT sequence as the primer.

6. The method of claim 5 wherein said oligo or poly dT sequence is at least about eight dT in length.

20 7. The method of claim 1 or 2 wherein said random primers are six nucleotides or longer in length.

8. The method of claim 7 wherein said random primers are nine nucleotides or longer in length.

25 9. The method of claim 1 or 2 wherein the bound nucleic acids are eluted in an elution volume of 25 microliters or less.

10. The method of claim 9 wherein said elution volume is 15 microliters or less.

5 11. The method of claim 1 or 2 wherein the promoter-primer complex of a) comprises a T7 promoter sequence.

12. The method of claim 1 or 2 wherein said solid phase is a filter.

10 13. The method of any one of claims 1, 2 or 12 wherein said solid phase comprises silica.

14. The method of claim 13 wherein said solid phase is glass powder, silica particles, glass fibers or microfibers, diatomaceous earth, or borosilicate glass.

15

15. The method of any one of the preceding claims wherein the wetting capacity of the solid phase is approximately the same as, or less than, the elution volume.

20 16. The method of any one of the preceding claims wherein said eluting of bound nucleic acids comprises centrifugation.

17. The method of claim 16 wherein said centrifugation is in two steps.

25 18. The method of claim 17 wherein said two steps comprise a first step and a second step at a higher speed than said first step.

19. The method of claim 16 wherein said centrifugation is without the application of a vacuum.

5 20. The method of claim 1 or 2 wherein a) and/or c) is conducted in a reaction time of 25 minutes or less.

21. The method of any one of the preceding claims wherein the amplified RNA is further amplified by a method comprising

10 e) reverse transcribing said amplified RNA using random primers and a DNA dependent polymerase for a reaction time of 45 minutes or less;

 f) producing a second strand cDNA complementary to said first strand cDNA using a second promoter-primer complex and a DNA dependent DNA polymerase for a reaction time of 45 minutes or less; and

15 g) producing re-amplified RNA from the double stranded cDNA by *in vitro* transcription using a DNA dependent RNA polymerase which initiates transcription from the primer of said promoter-primer complex;

 wherein after f) and before g) above, after g) above, or both, the product produced by f) or g) is purified by contacting said product with a solid phase which
20 binds nucleic acids followed by eluting bound nucleic acids from said solid phase in an elution volume of less than 50 microliters.

22. The method of claim 21 wherein the promoter-primer complex of f) comprises a T3 or SP6 promoter sequence.

25

23. The method of claim 21 wherein said random primers are six nucleotides or longer in length.

24. The method of claim 23 wherein said random primers are nine nucleotides or longer in length.

25. The method of claim 21 wherein said second promoter-primer complex
5 comprises a known primer sequence.

26. The method of claim 25 wherein said known primer sequence is complementary to the 3' region of said amplified RNA.

10 27. The method of claim 21, 25 or 26 wherein said second promoter-primer complex comprises a T3 or SP6 promoter region.

28. The method of claim 21 wherein the elution of bound nucleic acids after f) or g) is in an elution volume of 25 microliters or less.

15

29. The method of claim 28 wherein said elution volume is 15 microliters or less.

30. The method of any one of claims 21-29 wherein the solid phase used
20 after f) or g) is a filter.

31. The method of any one of claims 21-30 wherein said solid phase used after f) or g) comprises silica.

25 32. The method of claim 31 wherein said solid phase is glass powder, silica particles, glass fibers or microfibers, diatomaceous earth, or borosilicate glass.

33. The method of any one of claims 21-32 wherein the wetting capacity of the solid phase phase used after f) or g) is approximately the same as, or less than, the elution volume.

5

34. The method of any one of claims 21-32 wherein said eluting of bound nucleic acids comprises centrifugation.

35. The method of claim 34 wherein said centrifugation is in two steps.

10

36. The method of claim 35 wherein said two steps comprise a first step and a second step at a higher speed than said first step.

37. The method of claim 34 wherein said centrifugation is without the
15 application of a vacuum.

38. The method of claim 21 wherein e) and/or f) is conducted in a reaction time of 25 minutes or less.